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10/550,671	11/09/2005	Hideaki Yamaoka	TOYA114.007APC	1379

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EXAMINER	
LONG, SCOTT	

ART UNIT	PAPER NUMBER
1633	

NOTIFICATION DATE	DELIVERY MODE
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

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Office Action Summary	Application No.	Applicant(s)	
	10/550,671	YAMAOKA ET AL.	
	Examiner	Art Unit	
	SCOTT LONG	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 May 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-6,8 and 9* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-6 and 8-9* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

The examiner acknowledges receipt of Applicant's Remarks and Claim amendments, filed on 6 May 2009.

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 5-06-09 has been entered.

Claim Status

Claims 1-6 and 8-9 are pending. Claim 1 is amended. Claim 7 is canceled. Claims 1-6 and 8-9 are under current examination.

Priority

This application claims benefit as a 371 of PCT/JP04/04074 (filed 03/24/2004). This application claims benefit from foreign application JAPAN 2003-082739 (filed

03/25/2003). Accordingly, the instant application has been granted the benefit date, 25 March 2003, from JAPAN 2003-082739.

RESPONSE TO ARGUMENTS

35 U.S.C. 112, second paragraph

The rejection of claims 1-6 and 8-9 under 35 USC 112, 2nd paragraph is withdrawn in response to the applicant's claim amendments.

The applicant's claim amendments have been fully considered and are persuasive. The applicant has amended the claims to cancel the phrase "enhancing the expression of...glucose dehydrogenase" in claim 1. The examiner had indicated that this phrase contained the relative term "enhancing the expression." The claim amendment has overcome the instant rejection.

The applicant also amended the claims to cancel the phrase "improving expression of glucose dehydrogenase and providing high glucose dehydrogenase activity" in claim 1. The examiner had rejected the claims because the words, "improving" and "high" are relative terms which render the claim indefinite. The amended claims have cancelled these words from the claim. The claim amendment has overcome the instant rejection.

Therefore, the examiner hereby withdraws the rejection of claims 1-6 and 8-9 under 35 USC 112, 2nd paragraph.

35 USC 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-6 and 8-9 remain rejected under 35 U.S.C. 103(a) as being unpatentable over Sode (WO/2002/36779, published 10 May 2002) in view of Herbaud et al. (Biochim. Biophys Acta. 2000; Vol.1481(1): 18-24) as evidenced by Arslan et al. (Biochem. Biophys. Res. Commun. 251 (1998) 744-747) for the reasons of record and the comments below.

Applicant's arguments and claim amendments filed 6 May 2009 have been fully considered but they are not persuasive.

The applicant has amended instant claim 1 to recite "wherein the expression of a cytochrome c maturation system (ccm) and glucose dehydrogenase is enhanced compared to a wild strain or unmodified strain of *Escherichia* bacteria." The specification indicates "expression of a DNA encoding a glucose dehydrogenase complex of *Burkhorderia cepacia* could be improved in an Escherichia bacterium by enhancing the expression of the ccm system of the bacterium, and thus accomplished the present

invention" (page 2, lines 28-32). In addition, the claim amendments have specified the particular glucose dehydrogenase of *Burkhorderia cepacia* KS1.

The applicant indicates that the teachings of the cited art do not recite the claim limitations (page 3, last paragraph). Contrary to the applicant's assertion, Herbaud et al. teach "that when the ccm genes are provided on a plasmid together with the structural gene for a mono- and a diheme c-type cytochrome, the cytochrome maturation occurs and seems to be increased" (page 18, col.2). The cited art indicates that maturation of cytochrome C (i.e., α-subunit and the β-subunit of glucose dehydrogenase) was increased in *E.coli* when co-expressed with the ccm genes (Herbaud, page 18, col.2) and further teaches that as a result, "[t]he production of cytochrome c_3 (M_r 13,000) was increased by about 10%" (Herbaud, page 21, col.2, lines 3-5). Therefore, Herbaud et al. teach co-expression of the α-subunit and the β-subunit of glucose dehydrogenase from *D. vulgaris* in *E.coli* with genes of a ccm operon. Herbaud does not teach the α-subunit and the β-subunit of glucose dehydrogenase from *Burkhorderia cepacia* KS1. However, Sode et al. teach DNA encoding α-subunit, β-subunit, and γ-subunit (WO/2002/36779 Translation, lines 512-513, 592-595 and 722-724) of glucose dehydrogenase of *Burkhorderia cepacia* KS1 (Translation, lines 530-531) and Sode further teaches, plasmids including pBR322, pUC18, and pUC19 are suitable for expression of glucose dehydrogenase subunit genes in the host bacteria, *Escherichia coli* (Translation, lines 623-624). The cited references teach the claim limitations of the instant invention. Therefore, the examiner finds the applicant's arguments unpersuasive.

The applicant has provided the following reference: Sinha et al. (FEMS Microbiology Letters. 1998; 161: 1-6). The applicant interprets Sinha et al. as teaching away from the instant invention and thereby suggesting that the instant invention is non-obvious. In particular, the applicant states, "While the references taken together teach eh claim element, the combination is non-obvious in view of the teaching away reference submitted herewith (Sinha) and the unexpected high expression of GDH which has been discussed preciously and also below." (Remarks, page 5, lines 1-3). The examiner notes that this reference has not been used in the pending rejection. In addition, the examiner notes that Sinha teaches that "the maturation of *H. thermophilus* cytochrome c_{552} in the cytoplasm of *E. coli* is unique among bacterial c-type cytochromes" (abstract). The model of Sinha is not similar to the claimed invention because the GDH is identified as being "unique among bacterial c-type cytochromes." Therefore, the examiner concludes that the teachings of Sinha do not directly bear relevance to the claimed invention or the teachings of the references cited in the pending rejection. Accordingly the examiner finds the applicant's arguments unpersuasive.

The applicant proposes various arguments (Remarks, page 5, paragraphs 2-4) where the applicant attempts to compare the claimed invention with other microorganisms or systems which are not recited in the claims, as evidence of non-obviousness. The applicant discusses comparisons between a system with and without the ccm. This is not claimed and does not encompass the claimed system. The applicant discusses the GDH expression of Herbaud; Herbaud teaches using a GDH

from *D. vulgaris* rather than from *Burkhorderia cepacia* KS1. Therefore, the comparison is not a perfect match. Herbaud merely demonstrates the principle used by the instant inventors and Sode demonstrates the power of GDH from *Burkhorderia cepacia* KS1. None of the comparisons completely match the claimed microorganism. This is the difficulty with such an argument. The examiner cannot provide evidence of 100 times increase in expression. However, the examiner has shown that the art teaches the general principle of the claimed invention and the recited elements. Therefore, the examiner concludes there is a prima facie case of obviousness. The "unexpected" 100 times increase in cytochrome c activity, asserted by the applicant, would flow from assembling the recited elements. There is a clear suggestion for assembling the recited elements in an E. coli to achieve a generally "enhanced expression." Accordingly, the examiner finds the applicant's arguments unpersuasive.

The applicant asks that the argument (Remarks, filed 10/10/2008, page 6, lines 9-12) made in the previous Applicant's Arguments (filed 10/10/2008) be reconsidered. Apparently the examiner was not successful in explaining his point of view. The applicant argues, "in at least one instance stimulation of cytochrome c production by expression of ccm genes was not observed as inclusion of ccm with cytochrome c550 from *B. subtilis* did not produce any increase in production (see page 747, col.1, last paragraph)." First, Arslan teaches "Plasmid pEC86 provides a tool for constitutive *ccm* gene expression and in particular facilitates aerobic cytochrome *c* maturation. It can also be used to increase the amounts of endogenous *c*-type cytochromes." The section to which the applicant refers (page 747, col.1, last paragraph) states, "The *B. subtilis*

membrane-bound cytochrome *c*-550 is expressed and matured in aerobically grown *E. coli* without pEC86." The examiner cannot understand the applicant's argument since it is clear that the citation (page 747, col.1, last paragraph) does not refer to a modified microorganism as claimed or suggested by the cited art. The examiner included the Arslan reference because the limitations of claim 8 are directed to the Escherichia bacterium according to claim 1, wherein the plasmid is pEC86. Herbaud et al. teach using pEC86, but do not show the structure of this plasmid. Can the applicant please clarify how pointing to an E.coli without pEC86 demonstrates non-obviousness? In the last Action, the examiner found this to be irrelevant to the current discussion, since a bacterium without the required elements cannot show that a bacterium with the required elements does not work. The examiner finds his position is the same and the applicant's argument is unpersuasive. The examiner hopes his response is clearer than the last attempt.

Therefore, the examiner hereby maintains the rejection of claims 1-6 and 8-9 under 35 USC 103(a) as obvious over Sode in view of Herbaud et al. and as evidenced by Arslan et al.

The examiner reiterates the rejection of record (Action, filed 6/1/2007) below:

Claims 1-6 and 8-9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Sode (WO/2002/36779, published 10 May 2002) in view of Herbaud et al. (Biochim. Biophys Acta. 2000; Vol.1481(1): 18-24) as evidenced by Arslan et al. (Biochem. Biophys. Res. Commun. 251 (1998) 744-747).

Claim 1 is directed to an *Escherichia* bacterium, comprising DNAs encoding the α-subunit and the β-subunit of glucose dehydrogenase of *Burkhorderia cepacia* KS1 in an expressible form and further comprising genes of a ccm Operon operably linked to a promoter, wherein the expression of a cytochrome c maturation system (ccm) and glucose dehydrogenase is enhanced compared to a wild strain or unmodified strain of *Esherichia* bacteria. Sode et al. teach DNA encoding α-subunit, β-subunit, and γ-subunit (WO/2002/36779 Translation, lines 512-513, 592-595 and 722-724) of glucose dehydrogenase of *Burkhorderia cepacia* KS1 (Translation, lines 530-531). Sode teaches, plasmids including pBR322, pUC18, and pUC19 are suitable for expression of glucose dehydrogenase subunit genes in the host bacteria, *Escherichia coli* (Translation, lines 623-624). Intrinsically, Sode teaches constitutive expression of the glucose dehydrogenase, as suggested by the ability of Sode to produce the glucose dehydrogenase complex by merely culturing the transformed bacteria (Translation, lines 20-23). There is no mention of inducible promoters, so the examiner interprets the Sode reference as having constitutive expression of the glucose dehydrogenase subunits. According to the instant specification, the phrase "enhance the expression of the ccm system" is defined to mean recombinant glucose dehydrogenase genes constitutively expressed in *Escherichia* (Specification, page 9, parag.2).

Claim 2 is directed to the Escherichia bacterium according to claim 1, wherein the DNA encoding the α-subunit is located upstream from the DNA encoding the β-subunit, and expression of the subunits is regulated by a single promoter. Sode

teaches, expression plasmids comprising nucleic acid sequences wherein the alpha subunit is upstream of the beta subunit (lines 723-724).

Claims 3-4 are directed to the Escherichia bacterium according to claim 1, wherein the DNA encoding the γ-subunit is located upstream from the DNA encoding the α-subunit. Sode teaches, transformants comprising expression plasmids wherein the nucleic acid sequence for the gamma subunit is upstream of the alpha subunit (lines 1230-1233).

Claim 5 is directed to the Escherichia bacterium according to claim 1, wherein the Escherichia bacterium is Escherichia coli. Sode teaches transformation of E. coli with the plasmids comprising α-subunit, β-subunit, and γ-subunit of GDH.

Claim 6 is directed to a method for producing a glucose dehydrogenase complex, which comprises culturing the *Escherichia* bacterium according to claim 1 so that the DNAs encoding the α-subunit and the β-subunit are expressed and the glucose dehydrogenase complex is produced, and collecting the complex. Sode teaches, "The manufacture procedure of the glucose dehydrogenase characterized by belonging to *Burkhorderia cepacia*, cultivating to a medium the microbe which has the capability to produce glucose dehydrogenase, and extracting glucose dehydrogenase from this medium or/and said microbe cell." (Translation, lines 20-23).

Claim 8 is directed to the Escherichia bacterium according to claim 1, wherein the plasmid is pEC86.

Sode. does not teach the specific plasmid, pEC86.

Herbaud et al. teach E. coli transformed with "pEC86 that contains the ccm genes" (page 19, col.2), in particular, those encoding α-subunit, β-subunit, and γ-subunit. Herbaud also teach "when the ccm genes are provided on a plasmid together with the structural gene for a mono- and a diheme c-type cytochrome, the cytochrome maturation occurs and seems to be increased" (page 18, col.2).

Claim 9 is directed to the Escherichia bacterium according to claim 1, wherein the bacterium is modified so that the expression of the ccm system is enhanced by replacing the bacterium's ccm operon promoter with another promoter. Herbaud teaches the plasmid, pEC86. Herbaud et al. (page 19, Materials and Methods, section 2.1) indicate that Arslan et al. describe in greater detail the structure of pEC86. Arslan et al. teach, "Overproduction of c-type cytochromes with pEC86 encoding the *ccm* genes." (page 745, col.1, Results). Arslan et al. further teach, "Plasmid pEC86 is derived from the vector pACYC184 and contains the *ccm* genes downstream of the *tet* promoter." (page 745, col.2). In addition, Arslan et al. teach, "Plasmid pEC86 provides a tool for constitutive *ccm* gene expression and in particular facilitates aerobic cytochrome *c* maturation. It can also be used to increase the amounts of endogenous *c*-type cytochromes." (page 747, col.1).

Therefore, Herbaud et al. teach co-expression of the α-subunit and the β-subunit of glucose dehydrogenase from *D. vulgaris* in *E.coli* with genes of a ccm operon. Herbaud does not teach the α-subunit and the β-subunit of glucose dehydrogenase from *Burkhorderia cepacia* KS1. However, Sode et al. teach DNA encoding α-subunit, β-subunit, and γ-subunit (WO/2002/36779 Translation, lines 512-513, 592-595 and 722-

724) of glucose dehydrogenase of *Burkhorderia cepacia* KS1 (Translation, lines 530-531) and Sode further teaches, plasmids including pBR322, pUC18, and pUC19 are suitable for expression of glucose dehydrogenase subunit genes in the host bacteria, *Escherichia coli* (Translation, lines 623-624). Arslan and Herbaud teach that pEC86 contain the necessary genes for expressing ccm genes.

It would have been obvious to the person of ordinary skill in the art at the time the invention was made to utilize the specific plasmid, pEC86, as taught by Herbaud et al. with the invention of Sode.

The person of ordinary skill in the art would have been motivated to modify the teachings of Sode in with the teachings of Herbaud et al. because "when the ccm genes are provided on a plasmid together with the structural gene for a mono- and a diheme c-type cytochrome, the cytochrome maturation occurs and seems to be increased" (Herbaud et al., page 18, col.2).

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Sode and Herbaud et al. because each of these teachings generated enhancement of the ccm system.

Therefore the method as taught by Sode in view of Herbaud et al. and as evidenced by Arslan et al. would have been *prima facie* obvious over the method of the instant application.

Therefore, the examiner hereby maintains the rejection of claims 1-6 and 8-9 under 35 USC 103(a) as obvious over Sode in view of Herbaud et al. and as evidenced by Arslan et al.

Conclusion

No claims are allowed.

Examiner Contact Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Scott Long** whose telephone number is **571-272-9048**. The examiner can normally be reached on Monday - Friday, 9am - 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Joseph Woitach** can be reached on **571-272-0739**. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SDL/ Scott Long
Patent Examiner, Art Unit 1633

/Janet L. Epps-Smith/
Primary Examiner, Art Unit 1633